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TRANSLATION NO. 2567

DATE: Oct. 1964

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Obtaining concentrated non-infectious cultural antigens from viruses
of the tick-borne encephalitis group

Following is the translation of an article by V. S. Kokorev and S. F. Zakirova, Sverdlovsk Scientific-Research Institute of Viral Infections, published in the Russian-language periodical Laboratornoye Delo (Laboratory Practice) 6: 368-72, 1966. It was submitted on 31 Aug 1964.]

A great deal of attention is being given to problems of obtaining cultural antigens and using them for serological investigations in the case of tick-borne encephalitis (TE). Many investigators studied the dynamics of multiplication of viruses of tick-borne encephalitis in tissue cultures, hemagglutinating, complement fixing, and infectious properties of cultural antigens, the possibility of using them in the precipitation reaction, and problems of their inactivation and concentration [1-9].

In previous works we studied the dynamics of accumulation of tick-borne encephalitis virus in tissue cultures, especially its hemagglutination and infectious properties, conditions promoting the maximum accumulation of hemagglutinins in virus-infected tissue cultures, and also the possibility of using cultural antigens in the hemagglutination reaction (HR) and in the hemagglutination suppression reaction (HSR) [2, 10].

The purpose of the present work was obtaining non-infectious cultural antigens of viruses which were suitable for use in the HSR and the RDPA (reaction of diffusion precipitation in agar) in the case of tick-borne encephalitis.

In the tests we used 5 strains of virus from the tick-borne encephalitis group: Sofin, Absettarov, G-11338 (KFD - Kyasanur forest disease), OHF (Omsk hemorrhagic fever), I-40 (louping ill), and 6 transplanted strains of tissue cultures: SOTs, Detroit-6, HEp-2, PCh (cells from human embryo kidney), KPK (cells from kidney of a cow), and HeLa. The tissue cultures were incubated in separating flasks. For infection we used a 10% brain virus-containing suspension. The end concentration of virus during infection was 10^{-3} . The control were cell cultures which had received a 10% brain suspension from mice which were not infected with the virus of tick-borne encephalitis. After 15-20 minutes contact between the suspension containing the virus and the cell monolayer the support medium was introduced - medium No 199 with 4-5% normal bovine or horse serum. On the 7-9th day after infection of the cells the cultural fluid was poured off, centrifuged at 2000 rpm for 20 minutes, and preserved at 4° . Subsequently it was used for obtaining the antigen.

The HR was set up in the generally accepted manner, using an 0.5% suspension of goose erythrocytes in a phosphate buffer at 4°. In the HSR the antigens were investigated with homologous immune sera and with sera from convalescents. The HSR was set up with 8 E of hemagglutinin. [Translator's note. The use of the letter E has not been determined. Whether by coincidence or not this is the first letter in the Cyrillic word yedinitsa or unit.] Contact between the antigen-antibody complex was carried out for 2 hours at 4°. Results were considered in 40 minutes and again after 2 hours. Infecting capacity of cultural antigens was checked by the intracerebral infection of white mice with 10-fold dilutions.

The reaction of diffusion precipitation in agar was set up in the Ochterlony modification with the use of 1% Difco agar prepared in physiological solution (pH 7.2). Diffusion took place in a moist chamber at 37°. The results were calculated in 24 hours and again in 48 hours. In the reaction we used hyperimmune rabbit sera to the investigated strains, and as a control - the serum of rabbits which were immunized with a 10% suspension of normal brain of white mice. Also used in the reaction was specific gamma-globulin from the Tomsk Scientific-Research Institute of Vaccines and Sera. For a control we used antirabic gamma-globulin and native horse serum, and also the serum of white mice which had been immunized with a virus-containing suspension from the brain of suckling mice.

For inactivation and concentration of cultural antigens we made use of drying by the open method at 37, 24, and 4° (in specially adapted incubator, desiccators with anhydrous calcium chloride or concentrated sulfuric acid, cold chamber) in Petri dishes. Residual moisture was determined by additional drying up to a constant weight in a drying chamber at 105°.

The samples of cultural antigens which were subjected to drying were prepared on various tissue cultures and checked in the HR, the RDPA, and for infection ability. Prior to drying not one cultural antigen which was preserved in a refrigerator produced a positive result in the RDPA. Titers of hemagglutinins for these antigens were 1:32 - 1:2048 depending on the strain of tick-borne encephalitis virus and the type of tissue culture on which it was incubated.

Titers of antigen were found in the limits of 10^{-5} - 10^{-8} (LD_{50} for mice). For drying the antigens were poured into Petri dishes on the basis of 20-40 ml. At 37° the drying of such volumes took place in 48-72 hours, at 24° - 72-96 hours, and at 4° - up to 4-7 days. Residual moisture in the dried antigens in the first case comprised 2.7--4.7%, in the second - 5.4--7.8%, and in the third - 6.3--8.6%. After complete drying the antigen was diluted with 1-3 ml of borate buffer solution (pH 9.0); concentration of antigen increased by 10-40 times with the initial. Concentrated antigens were investigated in the HR, the RDPA, and for residual infection capacity. Drying of

cultural antigens at 37, 24, and 4° led to a sharp increase in the titers of hemagglutinins, the appearance of precipitinogens, and inactivation of the tick-borne encephalitis virus only at 37 and 24°; drying at 4° did not render the virus completely harmless (death of 40-50% of mice infected with non-diluted concentrated antigen).

Concentrated antigens were investigated in the HR in pH limits of 5.9--7.0. Maximum titers of hemagglutinins of such antigens were obtained at a pH of 6.2 for the reaction.

Table 1 presents the results of the drying of antigens of various strains of tick-borne encephalitis virus which were incubated on HEP-2 tissue culture, and in Table 2 - the results of drying of 2 antigens (Sofin and Absettarov strains), obtained following incubation of viruses on various tissue cultures: SOTs, HEP-2, PCh, KPK, Detroit-6, and HeLa. In all cases a similarity is noted: increase of titers of hemagglutinins, appearance of precipitinogens (see Tables 1 and 2), loss of infection capacity. At antigen concentrations of 10-40 times the titer of hemagglutinins increased by 8-32 times and more. The HR with control cultural fluids, which was set up at the same time, yielded a negative result.

Table 1

Characteristics of specific activity (at 37°) of various dry antigens during incubation of viruses in HEP-2 cells

(a) Штамм вируса	(b) Титр геммагглютинации		(c) Титр преципитации		(d) Титр инфекционности	
	до кон- центрации	после кон- центрации	до кон- центрации	после кон- центрации	до инакти- вации	после инакти- вации
(g) Софин	1:512	1:8192		1:32	10 ⁻⁵	
(h) ОГЛ	1:512	1:4096		1:8	10 ⁻⁵	
(i) G-11338 (КЛБ)	1:512	1:8192	(e) Не обна- ру- жен	1:16	10 ⁻⁷	(m) Не ин- фекци- онны
(j) Абсет- таров	1:1024	1:16 384		1:8	10 ⁻⁵	
(k) И-40	1:256	1:4096		1:16	10 ⁻⁷	

Key: (a) Strain of viruses; (b) Titer of hemagglutinins; (c) Titer of precipitation; (d) Titer of infection capacity; (e) before concentra-
tion; (f) after concentration; (ee) before inactivation; (ff) after
inactivation; (g) Sofin; (h) OHF; (i) G-11338 (KFD); (j) Absettarov;
(k) I-40; (l) Not detected; (m) Not infectious.

Precipitinogens were detected in dilutions up to 1:8 - 1:32. Four and five control passages on white mice, and also passages on tissue cultures (chick fibroblasts, SOTs, HeLa) with the subsequent infection of mice did not reveal an infection capacity for antigens dried at 37 and 24°.

Table 2

Characteristics of concentrated antigens (drying at 37°)

(a)	(b) Штамм вируса КЭ									
	(c) Софин					(d) Абсеттаров				
	(e) Титр гемматоглининов		(f) Титр инфекционнойности	(g) Титр гемматоглининов	(h) Степень концентрации	(i) Титр гемматоглининов		(j) Титр инфекционнойности	(k) Степень концентрации	(l) Титр инфекционнойности
	(m) до кон-центрации	(n) после кон-центрации				(o) до кон-центрации	(p) после кон-центрации			
НСр-2	1:512	1:8192	16	10 ⁻⁵		1:1024	1:16384	16	10 ⁻⁵	
(1) ПЧ	1:512	1:8192	16	10 ⁻⁸		1:1024	1:32768	32	10 ⁻⁵	
(m) Дет-роит-6	1:128	1:2048	16	10 ⁻⁵	(q) Не ин-фекционны	1:8	1:256	32	10 ⁻⁴	(r) Не ин-фекционны
(n) СОЦ	1:2048	1:16384	8	10 ⁻⁶		1:32	1:512	16	10 ⁻³	
(o) HeLa	1:1024	1:32768	32	10 ⁻⁶		1:128	1:2048	16	10 ⁻⁵	
(p) КПК	1:1042	1:32768	32	10 ⁻⁷		—	—	—	—	

Key: (a) Tissue culture; (b) Strain of TE virus; (c) Sofin; (d) Absettarov; (e) titer of hemagglutinins; (f) titer of infection capacity; (g) degree of concentration (times); (h) before concentration; (i) after concentration; (j) before inactivation; (k) after inactivation; (l) PCH; (m) Detroit-6; (n) SOTs; (o) HeLa; (p) KPK; (q) Not infectious.

As is known, cultural virus of tick-borne encephalitis in a cell-free medium is unstable, especially at 37°. Drying of antigen by the stated method leads to the denaturation of proteins, which in combination with the large concentration of salts of the medium and products of metabolism apparently also conditions a loss of infection capacity. These factors, however, do not exert a negative influence on the hemagglutinating and precipitating properties of cultural antigens.

The appearance of bands of precipitation during dynamic observation of samples was noted in 25-29 hours after the onset of drying; the intensity and clarity of the precipitation lines increased with the approach of the end of drying. During the determination of the

titers of concentrated antigens in the RDPA, besides the clearly expressed specific band of precipitation, sometimes a second band appeared - weakly expressed and non-specific, apparently caused by the presence of brain component in the antigen. Proof of the non-specificity of the second band was the detection of it (also not constantly) in control experiments; it disappeared during subsequent dilutions of antigens (Fig. 1, 2).

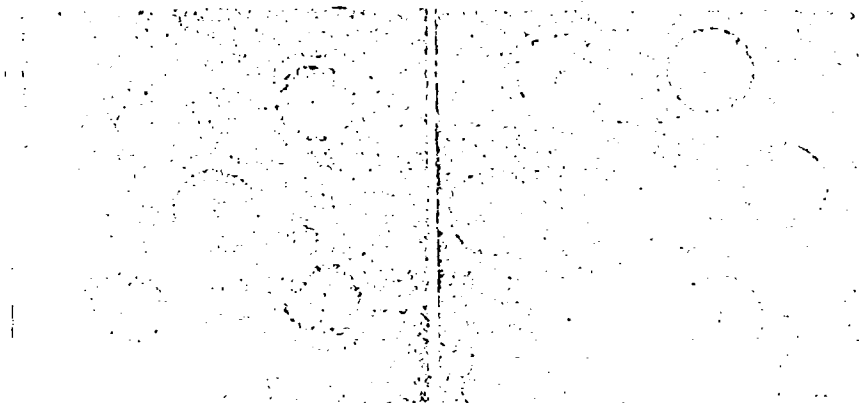


Fig. 1. Control test.
1-4 - cultural fluid of HEp-2 cells dried at 37°, concentrated (whole, 1:2, 1:4, 1:8);
5 - specific gamma-globulin in a dilution of 1:10.

Fig. 2. Test with antigen of the Sofin strain on HEp-2 tissue culture. Dried at 37°.
1-6 - concentrated antigen, dried at 37° (whole, 1:2, 1:4, 1:8, 1:16, 1:32);
7 - specific gamma-globulin in a dilution of 1:10.

The use of antirabic gamma-globulin and native horse serum in the control RDPA yielded negative results. In the event of using immune serum of white mice to the strains under investigation clear specific bands of precipitation were noted. Here there was little probability of a non-specific RDPA with antibodies to normal brain tissue of suckling mice ¹¹⁷. The results cited give a foundation to the consideration that the resulting antigens can be used in the RDPA.

The best results in respect to the detection of precipitinogens were noted during the concentration of antigens which were obtained on tissue cultures of HeLa, HEp-2, and KPK. Upon dilution of concentrated antigens to initial volumes precipitinogens were not revealed, and the titers of hemagglutinins were reduced to initial. Repeated dilution (3-4 times) with distilled water or borate buffer solution (pH 9.0) and drying of antigens did not bring about the loss or lowering of titers of hemagglutinins and precipitinogens, however, with subsequent storage they turned out to be somewhat less stable than with a one- or two-fold drying.

A check of the resulting antigens in the HSR with specific immune sera and sera of reconvalescents demonstrated their high degree of specific activity.

Storage of dried cultural antigens in a non-diluted state (in Petri dishes) at 37 and 24° for 4 months and dilution of them by borate buffer solution (pH 9.0) and storage for 7 months (period of observation) at 4° did not lead to a lowering of titers of hemagglutinins and precipitinogens.

The simplicity and accessibility of the described method make it possible to obtain non-infectious hemagglutinating and precipitating antigens of the tick-borne encephalitis virus in any virological laboratory.

Conclusions

1. Drying of cultural antigens for 48 hours and more by the open method at 37 and 24° causes the inactivation of the infection capacity of tick-borne encephalitis viruses and the concentration of hemagglutinins and precipitinogens. Ten-forty-multiple concentrations (as a result of dissolving dried antigens by 10-40 times with a lesser volume of solvent) produce an increase of titer of hemagglutinins by 8-32 times in comparison with initial and reveals precipitinogens with titers to 1:8 - 1:32.

2. The drying of cultural antigens of various strains of viruses of the tick-borne encephalitis group (Sofin, OHL, G-11338, Absettarov, I-40 strains), obtained on various tissue cultures (SOTs, HEp-2, Detroit-6, PCh, KPK, HeLa), produced monotypic results: concentration of hemagglutinins and precipitinogens and inactivation of infectious properties at 37 and 24°.

3. Optimum pH in the HR and the HSR for concentrated antigens equals 6.2 regardless of the strain of virus investigated and the type of tissue culture on which it is incubated.

4. Investigation of the resulting antigens in the HSR with specific immune sera and the sera of reconvalescents, and also in the RDPA with hyperimmune rabbit sera, immune mouse sera, and specific gamma-globulin showed their specificity in these reactions.

5. Storage of dried antigens in a non-diluted state (in Petri dishes) at 37 and 24° for 4 months and following their dilution with borate buffer solution (pH 9.0) and storage for 7 months (period of observation) at 4° did not cause a lowering of titers of hemagglutinins and precipitinogens.

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